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ARTICLE

Evaluation of Dietary Supplement Contamination by Xenobiotic and Essential Elements Using Microwave-Enhanced Sample Digestion and Inductively Coupled Plasma-Mass Spectrometry

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ABSTRACT. Dietary supplements were analyzed by evaluating the elemental content in six widely consumed products manufactured by four well-known companies. The elements included the neurotoxic and carcinogenic elements cadmium, mercury, aluminum, lead, arsenic, and antimony, as well as the essential elements zinc, selenium, chromium, iron, and copper, which were often not listed as ingredients on the product labels. Contamination from either xenobiotic or essential elements was found in all samples analyzed. The samples were prepared using US Environmental Protection Agency (EPA) Method 3052, microwave-enhanced digestion. The resulting digests were analyzed by Inductively Coupled Plasma-Mass Spectrometry based on EPA Method 6020B. The analytical protocols were validated by analyzing a multivitamin standard reference material, the National Institute of Standards and Technology Standard Reference Material 3280. The application of EPA standard methods demonstrated their utility in making accurate and precise measurements in complex matrices with multiple ingredients and excipients. In the future, the use of these methods could provide a uniform quality assurance protocol that can be implemented along with other industry guidelines to improve the production of dietary supplements.

KEYWORDS. dietary supplements, elemental contamination, EPA Method 3052, EPA Method 6020B, inductively coupled plasma-mass spectrometry, microwave-enhanced digestion

INTRODUCTION

Dietary supplements are the products containing vitamins, minerals, amino acids, plant materials, and/or other nutritional additives consumed to complement the human diet (Garcia-Rico et al., 2007). They are purchased by the public to resolve

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nutrient deficiencies, prevent birth defects, treat diseases and symptoms, and improve
athletic performance (Driscoll et al., 2010; Maughan, 2005; Raman et al., 2004). Supplements are widely available in retail stores, shopping malls, gyms, gas stations, convenience stores, and on the internet (Taylor, 2004). They continue to garner attention from media exposure from studies, articles, and advertisements (Radimer et al., 2004). The reasons for the increased popularity of dietary supplements include hope for the conservation of intellectual capacity, increased energy, stress relief, mitigation of high healthcare costs, and a perception that they contribute to a healthier lifestyle (Raman et al., 2004). Physicians routinely incorporate dietary supplements as part of their patient advice and clinical treatment. It was estimated that globally consumers had spent at least US$ 60 billion on dietary supplements in 2006 (Crowley & FitzGerald, 2006; Geyer et al., 2008), with a more recent estimate suggesting that between US$ 21 and 25 billion were spent annually in the United States alone (McCormick, 2010). Based on a National Health Interview Survey, over half the US adult population includes supplements in their daily diets (Cohen, 2009). The wide and growing consumption of dietary supplements makes it imperative for public health that existing quality control and assurance methods, some of which are decades old, are updated and streamlined utilizing advanced measurement tools that produce consistent, reliable, and quality data. Minimizing and avoiding unsafe levels of toxins and labeling errors require the quality control process to include the entire supply chain.

The expectations of the general public for dietary supplements are the same as they are for food: contribute nutrients and avoid any constituent that will have a negative health impact. Many reported instances of tainted products suggest that the public's expectations are not being met. It has been estimated that 50,000 unreported contamination incidences occur annually in dietary supplements in the United States (Cohen, 2009). These contaminants include heavy metals, bacteria, toxic plant matter, prescription medications, controlled substances, rejected drugs, and other compounds (Cohen, 2009).

The toxic effects of xenobiotic metals on human health have been well documented in the literature. These elements, including lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As), cause a wide variety of toxicological effects and symptoms affecting all organ systems, including the central nervous system (Neustadt & Pieczenik, 2007). Lead has irreversible, detrimental effects that interfere with neuronal signaling in the central nervous system, heme synthesis, and calcium (Ca) metabolism and function (Bourgoin et al., 1993; Sargent, 1994). Mercury exposure can cause kidney damage and neurological disorders (Chen et al., 2002). Cadmium results in reproductive disruption, kidney failure, and weakening of the bones (Garcia-Rico et al., 2007). Arsenic has been connected to hypertension, hypotension, cardiomyopathy, cardiac disease, and peripheral neuropathy (Jomova et al., 2011). Aluminum (Al) has been associated with Alzheimer's disease (Kawahara & Kato-Negishi, 2011).

In parallel with the negative effects of toxic heavy metals, there is concern about improper labeling of dietary supplements that omits the presence of essential elements, such as zinc (Zn), selenium (Se), chromium (Cr), iron (Fe), and copper (Cu). Although they may not be listed as supplement ingredients, their presence

can cause adverse health consequences, including the disruption of nutritional regimens. Adulteration related to these essential elements must be considered when evaluating dietary supplements for elemental contamination in addition to xenobiotic elements. Essential elements are common ingredients of dietary supplements because they are needed to maintain crucial metabolism in the body (Raghunath et al., 2006). Human metabolism requires many different metal coenzymes for the optimal functionality of numerous proteins. Although they are necessary, these elements can have negative consequences if present in excess. Zinc is required for the normal maturation and optimal performance of the immune system (Shankar & Prasad, 1998); however, patients that exceed the daily recommended allowance for Zn could experience leukopenia, neutropenia, anemia, sideroblastic anemia, and hypocupremia (Driscoll et al., 2010; Igic et al., 2002; Porea et al. 2000; Salzman et al. 2002). While Se exists naturally and is needed for proper cellular functioning (MacFarquhar et al., 2010), it is toxic at high levels, resulting in vomiting, nausea, pulmonary edema, renal and liver damage, and death (Fan & Kizer, 1990; Nuttall, 2006). Chromium performs a key function in the metabolism of carbohydrates and fats, and increases cellular response to insulin (Marrero et al., 2013); however, an overabundance can cause kidney failure, improper liver functioning, and gastrointestinal bleeding (MacFarquhar et al., 2010). Iron is well known for promoting oxygen transport and reducing oxygen shortages in cells, thereby preventing exhaustion and a compromised immune system (Marrero et al., 2013). Excess Fe can negatively affect the metabolism of other closely related elements such as Zn, Cu, and Ca, and may result in oxidative stress, digestive disturbances, and vomiting (Fairweather-Tait et al., 2011). Copper plays a role in many essential reactions needed for the survival of mammals (Linder & Hazegh-Azam, 1996), but Cu surplus can cause liver damage (Lopez de Romana et al., 2011). It has been hypothesized that the consumption of inorganic Cu in dietary supplements might contribute to the development of Alzheimer's disease (Brewer, 2009, 2011).

In spite of the known effects of xenobiotics, the regulation of dietary supplements has become less stringent in the United States after the passing of the Dietary Supplement Health and Education Act (DSHEA) in 1994 (Cohen, 2009) as a revision to the Federal Food, Drug, and Cosmetic Act (Saldanha et al., 2004). The testing requirements for the production and sale of dietary supplements were significantly relaxed in the DSHEA (Maughan, 2005). Dietary supplements were previously classified as being "food additives," which had mandated evidence of their safety before being released to consumers (Cohen, 2009). The DSHEA created a special category of food called "dietary supplements" (Saldanha et al., 2004), with provisions that were not applicable to conventional and functional foods (Taylor, 2004). Since dietary supplements do not diagnose, prevent, or cure disease according to the US Congress, they are not required to have the same regulatory standards as pharmaceutical drugs (Maughan et al., 2011). Subsequently, there is *no* US Food and Drug Administration (FDA)-required testing of dietary supplements for safety and efficacy prior to market release, and approval is not needed before the manufacture and distribution of these products (Ashar et al., 2007). Under the current guidelines, the FDA will only recommend testing after a dietary supplement has been determined to be unsafe (Woo, 2007). This relaxed regulatory requirement is

very different from the treatment of prescription drugs, which require FDA review of safety and effectiveness before reaching the market (Ashar et al., 2007). Without providing specific guidelines of dietary supplement production, the DSHEA established Good Manufacturing Practices (GMPs) to maintain the quality of dietary supplements available to the general public (Ashar et al., 2007). Extensive control over the entire manufacturing process is required for GMP compliance, including starting ingredients, finished goods, and quality control (Tumir et al., 2010). The DSHEA does not specify standard analytical methods, such as those from the US Environmental Protection Agency (EPA), which can be adopted and implemented for quality assurance as a way to improve public safety and attain GMP compliance. The US EPA is a globally recognized and respected repository of workgroup-tested and optimized methods, which may be adopted as templates and optimized as standard quality assurance and testing protocols.

Different government agencies have limits for exposure to certain elements referred to as maximum contaminant levels (MCLs), which are shown for each agency in Table 1. The United States Pharmacopeia (USP) has set elemental maximum values in dietary supplements for only inorganic As, Cd, Pb, total Hg, and methylmercury (United States Pharmacopeia, 2012b). This amount of regulation is much less stringent than what the USP requires for prescription drugs, where 15 elements are listed with concentration thresholds (United States Pharmacopeia, 2012a). The FDA does not have specific elemental limits for dietary supplements but rather focuses on GMPs to prevent the adulteration of dietary supplements from heavy metals, pesticides, and other harmful contaminants, and to ensure the presence of only the listed ingredients (Mindak et al., 2008). California Proposition 65 regulations provide a list of chemicals and compounds which are known to cause cancer or reproductive toxicity. Daily limits (Office of Environmental Health Hazard Assessment, 2012) and MCLs (Office of Environmental Health Hazard Assessment, 2012) are provided for some elements, but there is no specific information for dietary supplements. The EPA provides MCLs in drinking water, but the amount of drinking water consumed per day is typically much greater than the amount of dietary supplements ingested (US Environmental Protection Agency (EPA)). Recent papers investigating elemental contamination in dietary supplements have compiled tables with the advocated amounts of selected elements to be consumed each day (Avula et al., 2010, 2011). Commonly, these limits are calculated based on the average adult male; often they do not take into account women, children, and developing fetuses that can be more susceptible to the effects of these potentially harmful elements and chemicals. It is hard to assess the quality of dietary supplements based on the current guidelines provided by these regulatory agencies. However, the USP-published methods do not match the level of completeness and utility of the EPA methods as many USP methods are either too brief, inconsistent, or too old to be practical or useful today to achieve GMP compliance.

Numerous studies have demonstrated the problem of harmful elemental contamination in dietary supplements. Nearly 20 years ago, Pb was discovered in calcium supplements (Bourgoin et al., 1993), while a more recent study determined that two-thirds of calcium supplements contained Pb concentrations that exceeded the 1999 California limits (Scelfo & Flegal, 2000). Another study found wide concentration ranges for As, Cd, Hg, and Pb in dietary supplements (Dolan et al.,

TABLE 1. List of Inorganic Contaminants and Their MCLs by Regulatory Agency

Element	USP Dietary Supplement Limit ($\mu g\ g^{-1}$) (United States Pharmacopeia 2012b)	USP Drug Product Limit ($\mu g\ g^{-1}$) (United States Pharmacopeia 2012a)	Prop 65 NSRL[a] or MADL[b] ($\mu g\ day^{-1}$) (Office of Environmental Health Hazard Assessment)	Prop 65 MCL ($mg\ L^{-1}$) (Office of Environmental Health Hazard Assessment)	EPA Drinking Water MCL ($mg\ L^{-1}$) (United States Environmental Protection Agency (EPA))
Aluminum	No limit	No limit	No limit	1	0.05 to 0.2
Antimony	No limit	No limit	No limit	0.006	0.006
Arsenic	1.5	0.15	0.06 (inhalation) 10 (except inhalation)	0.01	0.010
Barium	No limit	No limit	No limit	1	2
Beryllium	No limit	No limit	0.1	0.004	0.004
Cadmium	0.5	2.5	0.05 (inhalation) 4.1 (oral)	0.005	0.005
Chromium (total)	No limit	No limit	No limit	No limit	0.100
Copper	No limit	100	No limit	1.3	1.3
Iridium	No limit	10	No limit	No limit	No limit
Iron	No limit	No limit	No limit	No limit	0.3
Lead	1.0	0.5	15 (oral) 0.5	0.015	0.015
Manganese	No limit	No limit	No limit	No limit	0.05
Mercury (Inorganic)	1.5	1.5	No limit	0.002	0.002
Molybdenum	No limit	10	No limit	No limit	No limit
Nickel	No limit	50	No limit	0.1	No limit
Osmium	No limit	10	No limit	No limit	No limit
Palladium	No limit	10	No limit	No limit	No limit
Platinum	No limit	10	No limit	No limit	No limit
Rhodium	No limit	10	No limit	No limit	No limit
Ruthenium	No limit	10	No limit	No limit	No limit
Selenium	No limit	No limit	No limit	No limit	0.05
Silver	No limit	No limit	No limit	No limit	0.10
Thallium	No limit	No limit	No limit	0.002	0.002
Uranium	No limit	No limit	No limit	25	0.030
Vanadium	No limit	10	No limit	No limit	No limit
Zinc	No limit	No limit	No limit	No limit	5

[a]NSRL: No significant risk level; [b]MADL: maximum allowable dose level.

2003). Studies from multiple countries noted xenobiotic and essential elemental contamination in natural drugs in China (Chuang et al., 2000), in herbal remedies in Nigeria (Obi et al., 2006), and in dietary supplements in Mexico (Garcia-Rico et al., 2007) and the United States (Avula et al., 2010, 2011).

Other researchers have investigated contamination from essential elements in dietary supplements. A recent study discussed widespread Se toxicity resulting from elevated Se quantities in a liquid dietary supplement that also contained a high concentration of Cr (MacFarquhar et al., 2010). Additional case studies have documented the toxicity caused by Se in dietary supplements (Sutter et al., 2008; Aldosary et al., 2012).

The project described in this paper started with an announcement from a US dietary supplement supplier that one of their supplements had been contaminated. This study expanded on the original discovery by evaluating elemental content in six common types of dietary supplements (zinc, essential fatty acids, calcium, magnesium, multivitamins, and probiotics) from four different manufacturers. The present study applied additional quality control parameters to the elemental analysis of dietary supplements through the use of the US Environmental Protection Agency (EPA) (1996) Method 3052 for sample preparation and the US Environmental Protection Agency (EPA) (2013) Method 6020B for analysis, with National Institute of Standards and Technology (NIST) Multivitamin/Multielement Tablets, Standard Reference Material (SRM) 3280, used for validation.

MATERIALS AND METHODS

Materials

Chemicals and Standards

All reagents were of analytical grade. ARISTAR ULTRA nitric acid (HNO_3), ARISTAR ULTRA hydrochloric acid (HCl), ARISTAR ULTRA water, borosilicate glass vessels, and 50-ml polypropylene centrifuge tubes were procured from VWR (Radnor, PA, USA). Multi-element and single element standards of Hg, iodine (I), lithium (Li), and yttrium (Y) were bought from Inorganic Ventures (Christiansburg, VA, USA). The tuning mix for the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) comprising 0.001-μg g^{-1} lithium, cobalt, yttrium, cerium, and tellurium in 2% (v:v) HNO_3 was purchased from Agilent Technologies (Santa Clara, CA, USA). The internal standard solution containing 100 μg g^{-1} of ^{6}lithium, scandium, germanium, rhodium, indium, terbium, lutetium, and bismuth was also obtained from Agilent Technologies. Ultra high purity grade (99.999%) liquid argon along with hydrogen and helium gases was procured from Airgas (Radnor, PA, USA). SRM 3280 was bought from NIST (Gaithersburg, MD, USA).

Dietary Supplement Samples

Six types of dietary supplements were purchased from four manufacturers in commercially available form (Table 2). From Company 1, zinc, calcium, and magnesium supplements were obtained. Zinc, essential fatty acids, calcium, magnesium,

and multivitamin supplements were tested from Company 2. All six supplement types were analyzed from Company 3 and 4.

Instrumentation

Sample preparation was accomplished by applying microwave-enhanced chemistry using a Milestone (Shelton, CT, USA) UltraWAVE laboratory microwave system equipped with temperature and pressure feedback control. This device accurately monitored and controlled temperature to within ±2°C of the specified values, automatically adjusting the microwave field output power to achieve the preset temperatures. The UltraWAVE allowed for extremely high temperature and pressure conditions of up to 300°C and 199 bar, respectively, to be maintained for extended periods during the digestion of the samples. This capability allowed for different types of samples to be digested simultaneously since all samples were held under the same conditions. In this study, borosilicate glass vessels were used for the digestion of 15 samples per batch.

Mass spectrometric analysis was performed using an Agilent 7700 ICP-MS with a micromist nebulizer, quartz spray chamber, an octopole reaction system, and a quadrupole mass analyzer. This instrument has a collision/reaction cell that uses hydrogen or helium gas for the elimination of polyatomic interferences. All samples and the internal standard were introduced into the ICP-MS using the peristaltic pump at a speed of 0.1 revolutions per second (rps) via the micromist nebulizer of the spray chamber. Before analysis, the samples were placed in a CETAC (Omaha, NE, USA) ASX-520 autosampler housed inside an ENC-500 anti-contamination enclosure. The ICP-MS and autosampler were located in an International Organization for Standardization Class 6 cleanroom laboratory. The optimized parameters of the ICP-MS and autosampler are shown in Table 3.

METHODS

Homogenization of the Dietary Supplements

Four different forms of dietary supplements were analyzed during this study: tablets, capsules, powders, and liquids. The procedure given in the Certificate of Analysis for NIST SRM 3280 was followed for sample homogenization. Fifteen SRM 3280 tablets were homogenized in a clean mortar and pestle and stored in a 50-ml polypropylene centrifuge tube. All of the tablet dietary supplement samples were prepared and stored in the same manner as SRM 3280. For each capsule sample, the outer casing was removed for 15 capsules and the inside contents were emptied into a 50-ml polypropylene centrifuge tube. For the powder samples, a quantity similar to 15 SRM 3280 tablets was poured into a 50-ml polypropylene centrifuge tube. The liquid samples were shaken immediately before being added to the microwave digestion vessels.

Microwave-Enhanced Digestion of the Dietary Supplements by EPA Method 3052

The homogenized samples were digested according to the EPA Method 3052 (Kingston et al., 1997; US Environmental Protection Agency (EPA), 1996) to determine their total elemental content. In each glass vessel, 0.25 g of sample was

TABLE 2. The Dietary Supplements Analyzed in the Study

Manufacturer	Supplement	Nutritional Ingredient Type	Other Ingredients
Company 1	Zinc	Mineral	Cellulose, water
	Calcium	Mineral	Ascorbyl palmitate, cellulose, water
	Magnesium	Mineral	Ascorbyl palmitate, cellulose, water
Company 2	Zinc	Mineral	Plant cellulose, stearic acid, rice flour, carob
	Essential fatty acid	Omega-3 fatty acids (docosahexaenoic acid, eicosapentaenoic acid)	Cholesterol, vitamins A, D_3, E, natural lemon flavor
	Calcium	Mineral	Fructose, sorbitol, stearic acid, magnesium stearate, vanilla, silicon dioxide
	Magnesium	Mineral	Gelatin capsule
	Multivitamin	Vitamins and minerals (vitamins A, C, D_3, E, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, biotin, pantothenic acid, calcium, iodine, magnesium, zinc, selenium, copper, manganese, chromium)	Microcrystalline cellulose, dicalcium phosphate, croscarmellose sodium, stearic acid, magnesium stearate, ethylcellulose, hydroxypropyl cellulose
Company 3	Zinc	Mineral	Vitamins C and B6, cellulose, vegetarian capsule, L-leucine
	Essential fatty acid	Fatty acid (marine oil concentrate, docosahexaenoic acid, eicosapentaenoic acid)	Natural mixed tocopherol vitamin E from soy, natural lemon oil, rosemary extract
	Calcium	Mineral	Phosphorus, hydroxypropyl methylcellulose, water, L-leucine, cellulose
	Magnesium	Mineral	Hydroxypropyl methylcellulose, water, L-leucine
	Multivitamin	Vitamins and minerals (vitamins A, C, D3, E, K1, thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, biotin, pantothenic acid, calcium, iodine, magnesium, zinc, selenium, manganese, chromium, molybdenum, potassium, boron, vanadium, choline, inositol, citrus bioflavonoids)	Natural citrus flavor
	Probiotic	Probiotic (probiotic blend, *lactobacillus acidophilus*, *bifidobacterium bifidum*)	Hydroxypropyl methylcellulose, water, L-leucine

Company 4	Zinc	Mineral	Purified water, sorbitol, glycerine, citric acid, natural raspberry flavor, potassium sorbate
	Essential fatty acid	Omega-3 fatty acid and vitamins (docosahexaenoic acid, eicosapentaenoic acid vitamin A, vitamin D)	Cod liver oil, purified water, gelatin, glycerine
	Calcium	Mineral	Calcium chelate, sodium starch, glycolate, magnesium stearate, vitamin D3, plant cellulose capsule
	Magnesium	Mineral	Magnesium glycinate, L-leucine, plant cellulose capsule
	Multivitamin	Vitamins and minerals (vitamins A, C, D, E, B1, B2, niacinamide, B6, folic acid, B12, biotin, pantothenic acid, calcium, iodine, magnesium, zinc, selenium, manganese, chromium, molybdenum, coenzyme Q10)	Dextrose, calcium carbonate, ascorbic acid, vitamin E acetate, magnesium oxide, zinc citrate, magnesium glycinate, calcium citrate, niacinamide, d-calcium pantothenate, manganese citrate, CoQ10, citric acid, natural peach mango flavor, starch, stearic acid, silicon dioxide, magnesium stearate, biotin, vitamin A acetate, pyridoxine, hydrochloride, riboflavin, thiamine mononitrate, glycerides of stearic and palmitic acids, mannitol, vitamin D-3, selenium chelate, di-calcium phosphate, beta carotene, cyanocobalamin, chromium chelate, molybdenum chelate, sucralose, folic acid, potassium iodide
	Probiotic	Probiotic (*lactobacillus rhamnosus*, *lactobacillus acidophilus*, *bifidobacterium bifidum/lactis*, *lactobacillus casei*, *lactobacillus plantarum*, *streptococcus therophilus*)	Stabilized probiotic strains, microcrystalline cellulose, silicon dioxide, magnesium stearate, plant cellulose capsule

TABLE 3. Optimized Operating Conditions for the Agilent 7700 ICP-MS for the Analysis of Dietary Supplement Samples According to EPA Method 6020B

Agilent 7700 ICP-MS Parameter	EPA Method 6020B Analysis Setting
Rf power	1,550 W
Spray chamber temperature	2°C
Acquisition mode	Spectrum
Peak pattern	3 points $mass^{-1}$
Sample depth	8 mm
Plasma gas	15 L min^{-1}
Carrier gas	0.9 L min^{-1}
Makeup gas	0.15 L min^{-1}
H_2 cell gas flow	4.5 ml min^{-1}
He cell gas flow	5.5 ml min^{-1}
Gas stabilization time	30 s
Integration time	0.1–1.0 s $mass^{-1}$
Replicates	4
Nebulizer pump speed	0.1 rps
Uptake time	30 s
Sabilization time	30 s

combined with 8-ml HNO_3 and 2-ml HCl. Three subsamples were prepared for each dietary supplement. The samples were digested in the microwave following a program that implemented a 10-min ramp to 180°C with a 10-min hold at 180°C. Complete digestion for all the types of dietary supplements (tablets, capsules, powders, and liquids) was achieved resulting in the production of homogeneous, transparent, light yellow solutions that did not contain any solids. After digestion, each sample was poured into a 50-ml polypropylene centrifuge tube and diluted with water to approximately 20 ml. The polypropylene centrifuge tubes were weighed before and after the addition of the sample digests. Six analytical blanks comprising HNO_3 and HCl were digested with the samples. The samples were stored in a cold room at 4°C and usually analyzed within three days of digestion. The microwave vessel caps were cleaned after digestion by soaking them in 1% HNO_3 overnight to prevent cross contamination.

Elemental Concentration Determination by ICP-MS Using EPA Method 6020B

The total concentrations of elements in the sample digests were determined according to the EPA Method 6020B (US Environmental Protection Agency (EPA), 2013). On each day of analysis, the system was aspirated with 2% HNO_3 for 30 min before tuning the instrument using the Agilent tuning solution at a concentration of 0.001 $\mu g\ g^{-1}$ and determining the pulse/analog factor. Analysis on the Agilent 7700 ICP-MS was performed in three modes: hydrogen, helium, and no gas. The amount of hydrogen and helium gas was optimized to eliminate polyatomic interferences. Table 4 lists the analysis mode for each element. Between analysis modes, a 30-s delay was employed to equilibrate the presence or absence of collision gas and/or reaction gas.

Due to the wide concentration range of the analytes of interest in SRM 3280 and the dietary supplement samples, calibration standards were prepared for 60 elements at three different concentration ranges (low, medium, and high) of 0–0.025,

TABLE 4. The Analysis Information, Correlation Coefficient, Detection Limits (DLs), and Background Equivalent Concentrations (BECs) for the Xenobiotic and Essential Elements of Interest Determined by the Agilent 7700 ICP-MS

Element	M/z	Mode	r^2	DL (ng g^{-1})	BEC (ng g^{-1})
Mg	24	He	0.9976	25.7	13.2
Al	27	H_2	0.9995	3.44	0.623
Ca	40	H_2	0.9983	110	93.9
V	51	He	0.9999	0.0537	0.0387
Cr	52	He	0.9999	0.961	3.76
Mn	55	He	1.0000	0.346	0.284
Fe	56	He	0.9985	18.8	15.4
Co	59	He	1.0000	0.0197	0.0438
Ni	60	He	0.9994	0.411	1.78
Cu	63	He	0.9999	0.309	0.684
Zn	66	He	0.9979	9.35	6.39
As	75	He	0.9999	0.0187	0.334
Se	78	H_2	1.0000	0.0178	0.0232
Mo	95	He	1.0000	0.102	0.334
Cd	111	He	1.0000	0.0477	0.0599
Sn	118	He	0.9999	0.105	0.295
Sb	121	No gas	0.9998	0.0866	0.130
Hg	201	No gas	1.0000	0.0658	0.0246
Pb	208	No gas	0.9999	0.00721	0.0752
U	238	No gas	1.0000	0.000834	0.00175

0–0.050, and 0–0.150 μg g^{-1}, respectively. The calibration standards were prepared by acid matching, which consisted of adding the appropriate percentage of microwave-digested reagent blank to each calibration standard.

The sample digests were diluted with water to total factors of 320, 8,000, and 800,000 for the low, medium, and high calibration ranges, respectively. These were analyzed in three separate sequences with four replicates taken from each subsample. Analyses were performed in spectrum mode using three points per mass. The integration time for each element ranged from 0.1–1.0 s per point depending on the element's ionization energy. The measured concentration value for each element in SRM 3280 and each dietary supplement sample was determined based on the value that fell within the range of one of the three calibration curves. After analysis was completed, the data were exported from the Agilent MassHunter software (version G7201A A.01.01) into Microsoft Excel for further data analysis.

Quality Control

The current study implemented numerous quality control measures mentioned previously that included the preparation of multiple subsamples, replicate analysis, preparation of analytical blanks, acid-matched calibration standards, and SRM 3280. It also utilized many of the measures described in the EPA Method 6020B (US Environmental Protection Agency (EPA), 2013.) An Agilent internal standard solution was used at a concentration of 1 μg g^{-1} in 1% HNO_3 and 0.5% HCl, and its acceptable recovery was maintained between 80 and 120% of the calibration blank. Between each sample, the autosampler probe was washed in three solutions of 1% HNO_3 and 0.5% HCl for 30 s each with a nebulizer pump speed of 0.5 rps.

After every nine samples, low- and mid-point calibration checks along with an SRM 3280 assessment were performed for additional calibration verification to ensure the consistency of the results during the complete duration of the sample run.

RESULTS

Analytical Figures of Merit and Validation of EPA Methods

All elements displayed linearity on the six- or seven-point external calibration curves for each of the three calibration ranges (Table 4). They had correlation coefficient (r^2) values greater than the 0.998 specified in the EPA Method 6020B except for magnesium (Mg, 0.9976) and Zn (0.9979) being just below the cutoff value only for the low calibration range. When rounded to three significant figures, they meet the specified requirements of the EPA Method 6020B. The four replicates for each calibration point displayed a variation of less than 5%, indicating that the obtained measurements were highly reproducible.

The elemental detection limits (DL) were defined as the average of the blank concentration for each element plus three times the standard deviation of the blank measurements for that element (Skoog et al., 2007). The DLs for all the elements in Table 4 were below 0.001 $\mu g\ g^{-1}$ except for Al, Ca, Fe, Mg, and Zn. The background equivalent concentrations (BECs) were calculated by multiplying the background intensity signal and the concentration of the analyte and dividing by the difference of the analyte intensity minus the intensity of the background (Thomas, 2008). The BECs were under 0.001 $\mu g\ g^{-1}$ for all elements other than Ca, Cr, Fe, Mg, Ni, and Zn (Table 4).

Additional confidence in the accuracy and precision of the results of the present study was achieved by using SRM 3280 to validate the sample preparation and analysis protocols. This SRM is certified for both xenobiotic and essential elements with their concentrations representing the wide range of values seen in commercially available products. Results obtained for SRM 3280 during this study are shown in Table 5 with the mean ($n = 12$) and 95% confidence intervals (CI). The measured values are in strong agreement with the certified and reference values, within 10% for nearly every element. The measured values overlapped the certified or reference CIs for 14 elements with the intervals for two other elements nearly intersecting, which is the same number of elements provided in two other studies (Avula et al., 2010, 2011). The recovery efficiencies ranged from 68.2 to 124% based on a comparison of the mean-listed values to the average measured quantities. Only three of the 23 certified or reference elements had either recoveries that differed by more than 20% from the mean value or that did not overlap CI values. This study provided measured values for the certified elements As, Cd, and Pb in SRM 3280 that were not included in two other studies (Avula et al., 2010, 2011). The value for As fell within the provided CI value, while Cd had a 118% recovery and Pb had a 70.7% recovery. The accuracy of all measurements in this study improved with the inclusion of the provided confidence intervals.

The precision determined by the 95% CIs was within 10% of the measured mean values for all of the certified or reference elements in SRM 3280, with over half inside 5% (Table 5). This level of precision for SRM 3280 was maintained for the

TABLE 5. Concentrations of All the Certified and Reference Elements Determined in SRM 3280 ($N = 12$, 95% CIs)

Element	Certified Value (μg g^{-1})	Measured Value (μg g^{-1})
B	141 ± 7	121.9 ± 4.7
Mg	$67,800 \pm 4,000$	$72,600 \pm 2,320$
P	$75,700 \pm 3,200$	$80,390 \pm 1,640$
Cl	$53,000 \pm 2,300$	Not Determined
K	$53,100 \pm 7,000$	$65,670 \pm 4,370$
Ca	$110,700 \pm 5,300$	$108,400 \pm 8,300$
Cr	93.7 ± 2.7	96.09 ± 2.03
Mn	$1,440 \pm 110$	$1,627 \pm 161$
Fe	$12,350 \pm 910$	$11,950 \pm 170$
Ni	8.43 ± 0.30	5.861 ± 0.299
Cu	$1,400 \pm 170$	$1,591 \pm 117$
Zn	$10,150 \pm 810$	$11,540 \pm 430$
As	0.132 ± 0.044	0.1625 ± 0.0138
Se	17.42 ± 0.45	17.98 ± 0.37
Mo	70.7 ± 4.5	84.12 ± 1.16
Cd	0.08015 ± 0.00086	0.09579 ± 0.00828
I	132.7 ± 6.6	Not Determined
Pb	0.2727 ± 0.0024	0.1973 ± 0.0077
	Reference Value (μg g^{-1})	Measured Value (μg g^{-1})
Na	330 ± 20	301.7 ± 16.7
Si	$2,010 \pm 10$	Not Determined
Ti	$5,400 \pm 300$	Not Determined
V	8 ± 2	9.691 ± 0.635
Co	0.81 ± 0.01	0.8572 ± 0.0167
Sr	29.8 ± 0.2	29.96 ± 1.24
Sn	11.1 ± 0.9	11.74 ± 0.62
Sb	0.159 ± 0.008	0.1085 ± 0.0057
La	0.70 ± 0.01	0.6151 ± 0.0118

remainder of the study as most results had 95% CIs that were less than 10% of their mean value. A large number of these were lower than 5% and some approached 1%. The other two studies (Avula et al., 2010, 2011), which have evaluated elemental content in botanicals and dietary supplements, supplied no standard deviation or confidence interval information for their sample measurements, listing only standard deviations for their SRM 3280 results. The exclusion of standard deviation and confidence intervals provided no information on their ability to sustain precise measurements for their samples during the remainder of their studies. This study provided 95% CIs for all detectable elemental quantities in the dietary supplement samples.

Determination of Elemental Concentrations in Dietary Supplements Using EPA Method 6020B

The elemental analysis results from the four dietary supplement companies are shown in Tables 6–9, respectively. The results are presented by company and separated into xenobiotic and essential elements. Only the most important results will be described in the text form. Contamination from essential elements was considered to exist if that element was not listed as an ingredient on the label for that particular dietary supplement.

TABLE 6. Concentrations (μg g^{-1}) of Xenobiotic and Essential Elements Determined in Dietary Supplements from Company 1 ($N = 12$, 95% CIs)

	Zinc Supplement	Calcium Supplement	Magnesium Supplement
Xenobiotic Elements			
Al	<DL	<DL	<DL
As	<DL	<DL	<DL
Cd	0.1168 ± 0.0089	<DL	<DL
Hg	<DL	<DL	<DL
Pb	<DL	0.1718 ± 0.0032	0.009087 ± 0.001802
Sb	<DL	<DL	<DL
Sn	0.02260 ± 0.00895	0.7526 ± 0.0355	0.02330 ± 0.00553
U	<DL	0.0009512 ± 0.0001482	0.004564 ± 0.000150
V	<DL	0.02266 ± 0.00509	2.129 ± 0.050
Essential Elements			
Ca	<DL	**257,500 ± 8,600**	208.3 ± 14.3
Co	<DL	<DL	0.05117 ± 0.00401
Cr	<DL	<DL	2.430 ± 0.119
Cu	<DL	0.3349 ± 0.0236	0.2421 ± 0.0130
Fe	<DL	7.974 ± 1.439	15.81 ± 1.63
Mg	<DL	5,578 ± 807	**138,900 ± 4,800**
Mn	<DL	0.3570 ± 0.0306	3.192 ± 0.133
Mo	<DL	0.05454 ± 0.01049	0.5963 ± 0.0210
Ni	<DL	<DL	1.774 ± 0.073
Se	<DL	0.04391 ± 0.00265	0.02158 ± 0.00451
Zn	**94,200 ± 1,100**	83.28 ± 2.81	<DL

<DL: Below detection limit.
Elements in bold are not considered elemental contamination due to their inclusion in the ingredient list.

Xenobiotic Elements

The supplements from Company 1 were found to have less contamination from xenobiotic elements compared with the other companies (Table 6). The calcium supplement contained tin (Sn) at a concentration of over 0.7 μg g^{-1} and Pb above 0.17 μg g^{-1}. The magnesium supplement had a similar frequency of contamination with vanadium (V) found at more than 2 μg g^{-1} along with Sn and Pb. Most notably, the zinc supplement had 0.117 μg g^{-1} of Cd.

Similar results were found in the dietary supplements analyzed from Company 2 with a high frequency of contamination by xenobiotic elements (Table 7). The concentration of Al in the magnesium, zinc, and multivitamin supplements was determined to be more than 10 μg g^{-1}. The magnesium supplement also contained more than 7.8 μg g^{-1} of V. Except for the essential fatty acids supplement, Pb contamination was observed in all supplements, with the zinc supplement having a concentration of 0.738 μg g^{-1}, the highest of the dietary supplements tested in this study. The multivitamin supplement had the highest amount of contamination from xenobiotic elements that included As, Cd, antimony (Sb), Sn, U, and V. The zinc supplement contained Cd, Sb, Sn, and V. The calcium supplement was sullied with V and As. The essential fatty acids supplement was not contaminated with any xenobiotic elements.

Among the products tested, Company 3 had the highest frequency of xenobiotic contamination in its products (Table 8). The calcium, magnesium, zinc, and multivitamin supplements contained high amounts of Al with the mean concentra-

TABLE 7. Concentrations (μg g^{-1}) of Xenobiotic and Essential Elements Determined in Dietary Supplements from Company 2 ($N = 12$, 95% CIs)

	Zinc Supplement	Essential Fatty Acids Supplement	Calcium Supplement	Magnesium Supplement	Multivitamin Supplement
Xenobiotic Elements					
Al	10.75 ± 2.92	<DL	<DL	61.07 ± 15.91	12.20 ± 3.00
As	<DL	<DL	0.01535 ± 0.00353	0.04374 ± 0.00499	0.1484 ± 0.0125
Cd	0.07024 ± 0.01027	<DL	<DL	<DL	0.07948 ± 0.00890
Hg	<DL	<DL	<DL	<DL	<DL
Pb	0.7384 ± 0.0193	<DL	0.03124 ± 0.00598	0.05145 ± 0.00232	0.2479 ± 0.0097
Sb	0.01004 ± 0.00166	<DL	<DL	<DL	0.05058 ± 0.00363
Sn	0.04709 ± 0.00619	<DL	<DL	<DL	0.09608 ± 0.01440
U	0.003061 ± 0.000240	<DL	0.004006 ± 0.000166	0.007497 ± 0.000349	0.3710 ± 0.0159
V	0.03149 ± 0.00577	<DL	0.05367 ± 0.00458	7.849 ± 0.114	0.7924 ± 0.0327
Essential Elements					
Ca	547.3 ± 76.6	<DL	**85,970 ± 1,500**	815.1 ± 56.7	**43,210 ± 2,340**
Co	0.009075 ± 0.002813	<DL	<DL	0.1777 ± 0.0081	1.015 ± 0.069
Cr	0.3442 ± 0.0370	<DL	<DL	5.971 ± 0.332	**97.07 ± 2.34**
Cu	3.853 ± 1.519	0.7182 ± 0.2176	<DL	0.5375 ± 0.0933	**3,830 ± 193**
Fe	26.21 ± 0.83	<DL	0.7639 ± 0.2146	54.64 ± 1.89	40.27 ± 7.52
Mg	318.8 ± 17.2	14.46 ± 5.84	855.0 ± 14.8	**634,600 ± 6,248**	**73,190 ± 3,860**
Mn	1.080 ± 0.130	0.5131 ± 0.2153	4.855 ± 1.137	12.20 ± 0.81	**3,226 ± 280**
Mo	0.1232 ± 0.0106	<DL	<DL	2.154 ± 0.052	0.5949 ± 0.0483
Ni	<DL	<DL	<DL	6.153 ± 0.214	0.4588 ± 0.1118
Se	<DL	0.01464 ± 0.00514	<DL	0.05102 ± 0.00408	**51.20 ± 3.05**
Zn	**79,120 ± 1,710**	25.800 ± 13.580	<DL	<DL	**29,070 ± 1,380**

<DL: Below detection limit.
Elements in bold are not considered elemental contamination due to their inclusion in the ingredient list.

TABLE 8. Concentrations (μg g^{-1}) of Xenobiotic and Essential Elements Determined in Dietary Supplements from Company 3 ($N = 12$, 95% CIs)

	Zinc Supplement	Essential Fatty Acids Supplement	Calcium Supplement	Magnesium Supplement	Multivitamin Supplement	Probiotic Supplement
Xenobiotic Elements						
Al	15.44 ± 6.29	<DL	26.09 ± 14.17	31.93 ± 4.82	20.88 ± 2.89	<DL
As	0.01153 ± 0.00367	<DL	0.02067 ± 0.00647	0.4323 ± 0.0221	0.5132 ± 0.0238	0.04750 ± 0.00780
Cd	0.03946 ± 0.00618	<DL	0.02777 ± 0.00334	0.06962 ± 0.00489	0.05674 ± 0.00992	<DL
Hg	<DL	<DL	<DL	<DL	<DL	<DL
Pb	0.4092 ± 0.0074	<DL	0.09268 ± 0.00328	0.02407 ± 0.00081	0.04092 ± 0.00155	0.05856 ± 0.00201
Sb	<DL	<DL	<DL	0.02754 ± 0.00169	<DL	<DL
Sn	0.1041 ± 0.0131	<DL	<DL	0.01445 ± 0.00362	0.4440 ± 0.0260	0.02863 ± 0.01034
U	0.0007583 ± 0.0001011	<DL	0.08226 ± 0.00141	0.5130 ± 0.0052	0.1032 ± 0.0017	0.02199 ± 0.00032
V	0.01963 ± 0.00728	<DL	0.3309 ± 0.0123	2.833 ± 0.062	11.66 ± 3.27	0.01567 ± 0.00683
Essential Elements						
Ca	87.87 ± 2.78	<DL	**299,000 ± 79,600**	1, 150 ± 80	**43,000 ± 740**	614.9 ± 235.3
Co	<DL	<DL	<DL	0.02499 ± 0.00344	0.4791 ± 0.0120	0.07519 ± 0.00497
Cr	<DL	<DL	<DL	1.530 ± 0.091	**27.21 ± 0.68**	<DL
Cu	0.1921 ± 0.0192	<DL	0.1949 ± 0.0535	0.5806 ± 0.0259	0.5046 ± 0.0392	0.9184 ± 0.0264
Fe	<DL	<DL	21.18 ± 3.19	44.97 ± 1.02	43.61 ± 2.28	3.924 ± 0.802
Mg	33.21 ± 3.68	2.480 ± 0.326	2, 423 ± 41	**179,400 ± 3,200**	**63,310 ± 1,830**	708.5 ± 16.6
Mn	1.206 ± 0.059	<DL	3.539 ± 0.090	7.663 ± 0.246	**360.8 ± 4.8**	293.5 ± 4.4
Mo	<DL	<DL	0.09942 ± 0.00604	1.448 ± 0.065	**18.30 ± 0.66**	0.08321 ± 0.02328
Ni	<DL	<DL	<DL	0.8782 ± 0.0715	0.4698 ± 0.0798	<DL
Se	<DL	0.008819 ± 0.001073	0.02959 ± 0.00397	<DL	**50.80 ± 2.73**	0.01847 ± 0.00143
Zn	**72.08 ± 1.02**	<DL	38.69 ± 1.67	4.337 ± 0.233	**2,839 ± 123**	24.62 ± 2.53

<DL: Below detection limit.
Elements in bold are not considered elemental contamination due to their inclusion in the ingredient list.

TABLE 9. Concentrations (μg g^{-1}) of Xenobiotic and Essential Elements Determined in Dietary Supplements from Company 4 ($N = 12$, 95% CIs)

	Zinc Supplement	Essential Fatty Acids Supplement	Calcium Supplement	Magnesium Supplement	Multivitamin Supplement	Probiotic Supplement
Xenobiotic Elements						
Al	<DL	3.424 ± 1.208	91.74 ± 3.42	<DL	31.22 ± 3.050	<DL
As	<DL	0.007569 ± 0.002793	0.2096 ± 0.0125	0.01163 ± 0.00413	0.09037 ± 0.00767	0.02217 ± 0.00572
Cd	<DL	<DL	0.1194 ± 0.0077	<DL	0.03310 ± 0.00605	<DL
Hg	<DL	<DL	<DL	<DL	<DL	<DL
Pb	0.003119 ± 0.000568	<DL	0.05814 ± 0.00626	<DL	0.06468 ± 0.00132	<DL
Sb	14.85 ± 0.15	<DL	<DL	<DL	<DL	<DL
Sn	<DL	<DL	<DL	0.03139 ± 0.00902	<DL	<DL
U	<DL	<DL	0.3197 ± 0.0057	0.004984 ± 0.000197	0.08917 ± 0.00175	0.01587 ± 0.00036
V	<DL	0.007211 ± 0.002768	1.719 ± 0.035	0.8498 ± 0.0284	0.5660 ± 0.0126	0.01093 ± 0.00424
Essential Elements						
Ca	<DL	6.153 ± 1.613	**242,900 ± 4,100**	198.7 ± 24.2	**16,760 ± 1,230**	73.11 ± 18.88
Co	<DL	<DL	0.03813 ± 0.00433	0.05813 ± 0.00423	0.2548 ± 0.0055	0.05438 ± 0.00386
Cr	<DL	<DL	2.430 ± 0.201	2.813 ± 0.096	**14.32 ± 0.42**	<DL
Cu	<DL	<DL	0.2673 ± 0.0250	0.1516 ± 0.0153	0.08997 ± 0.01458	0.3461 ± 0.0134
Fe	<DL	<DL	133.2 ± 2.8	29.04 ± 1.26	39.62 ± 1.22	<DL
Mg	<DL	5.518 ± 0.709	1, 459 ± 23	**196,500 ± 4,300**	**19,020 ± 970**	1, 440 ± 30
Mn	<DL	<DL	44.68 ± 1.31	4.370 ± 0.147	**920.2 ± 60.5**	221.0 ± 3.5
Mo	<DL	<DL	<DL	0.6047 ± 0.0329	**9.774 ± 0.742**	<DL
Ni	<DL	<DL	<DL	1.164 ± 0.062	<DL	<DL
Se	<DL	0.02422 ± 0.00209	0.006817 ± 0.001101	0.01070 ± 0.00145	**12.44 ± 1.39**	0.01334 ± 0.00159
Zn	**2,829 ± 81**	<DL	4.298 ± 0.260	<DL	**6,323 ± 220**	10.65 ± 0.23

<DL: Below detection limit.
Elements in bold are not considered elemental contamination due to their inclusion in the ingredient list.

tion of each more than 15 μg g^{-1}. Except for the essential fatty acids supplement, Pb was quantified in all of the samples with the zinc supplement having the highest concentration at nearly 0.41 μg g^{-1}. Of all the supplements tested, the multivitamin supplement had the highest concentration of As at 0.513 μg g^{-1}, and the magnesium supplement contained nearly as much As at 0.432 μg g^{-1} along with 0.444 μg g^{-1} of Sn. In both calcium and zinc supplements, elemental contamination from V, As, and Cd was found. The probiotic had low concentrations of V, As, Sn, and U. The essential fatty acids supplement was clear of contamination from xenobiotic elements with values below the respective detection limits.

Similar to both Companies 2 and 3, Al was a common contaminant among products of Company 4, as it was found at nearly 100 μg g^{-1} in the calcium supplement, over 30 μg g^{-1} in the multivitamin supplement, and above 3 μg g^{-1} in the essential fatty acids supplement (Table 9). The zinc supplement contained nearly 15 μg g^{-1} of Sb. In the calcium supplement, V was quantified at over 1.7 μg g^{-1} along with determining the presence of As, Cd, and U. Vanadium was found at 0.85 μg g^{-1} in the magnesium supplement in addition to As, Sn, and U. An elevated V amount was also observed in the multivitamin supplement at 0.566 μg g^{-1} along with As, Cd, Pb, and U. As and U were found in the probiotic supplement.

Essential Elements

In addition to the presence of xenobiotic elements, each dietary supplement from Company 1 had notable contamination by essential elements that were not listed as ingredients on the dietary supplement labels (Table 6). Overall, the calcium and magnesium supplements contained higher quantities of unlabeled essential elements compared with xenobiotic elements. In the calcium supplement, Mg was found at a concentration of over 5,000 μg g^{-1}, Zn at more than 80 μg g^{-1}, and Fe at 8 μg g^{-1}. The amount of contamination in the magnesium supplement consisted of Ca quantified at 200 μg g^{-1} and Fe at about 15 μg g^{-1} along with the presence of Cr, manganese (Mn), and nickel (Ni). In contrast, the zinc supplement contained no detectable quantities of any essential element.

Similar to Company 1, essential elements not listed as ingredients were found at a high frequency in the dietary supplements from Company 2 (Table 7). Magnesium was quantified at 855 μg g^{-1} in the calcium supplement and 318 μg g^{-1} in the zinc supplement. Calcium was found at 815 and 547 μg g^{-1} in the magnesium and zinc supplements, respectively. Except for the essential fatty acids supplement, Fe was observed in all the samples with the highest concentration of 54 μg g^{-1} in the magnesium supplement. The magnesium supplement was also determined to contain Cr, Mn, molybdenum (Mo), and Ni at concentrations between 2.1 and 12.2 μg g^{-1}. The calcium supplement had a Mn concentration of nearly 5 μg g^{-1}. Cobalt (Co), Mo, and Ni were detected in the multivitamin supplement. The zinc supplement frequently contained essential elements, notably Cu, Mn, and Cr. The presence of Zn and Mg was discovered in the essential fatty acids supplement.

The products from Company 3 also had contamination from unlabeled essential elements (Table 8). There was nearly 300 μg g^{-1} of Mn in the probiotic supplement. Magnesium was quantified at 2,500, 700, 33, and 2.5 μg g^{-1} in the calcium, probiotic, zinc, and the essential fatty acids supplements, respectively. In the magnesium and

zinc supplements, 1,150 and 88 μg g^{-1} Ca was measured, respectively. Zinc was also found in the calcium, magnesium, and probiotic supplements. Iron was detected in the magnesium, multivitamin, calcium, and probiotic supplements. Manganese was observed in the magnesium, calcium, and zinc supplements. Copper was found in the calcium, magnesium, zinc, and probiotic supplements. The magnesium supplement also had Cr, Ni, and Co. In addition, the multivitamin supplement contained Co and Ni.

As with the other three companies, the dietary supplements from Company 4 also contained contamination from essential elements (Table 9). Similar to Company 3, the probiotic supplement from Company 4 had a Mn concentration close to 250 μg g^{-1}. Magnesium was quantified at greater than 1,000 μg g^{-1} in the calcium and probiotic supplements. Calcium was measured at 198 and 73 μg g^{-1} in the magnesium and probiotic supplements. Of all the essential fatty acid supplements, the product from Company 4 was the most contaminated with essential elements, including Ca and Mg. In the probiotic and calcium supplements, Zn was quantified at 10 and 4 μg g^{-1}, respectively. Iron was found in the calcium, multivitamin, and magnesium supplements. In the calcium supplement, the Mn concentration was determined to be more than 40 μg g^{-1} with Cr at 2.4 μg g^{-1}. The magnesium supplement had Cr, Mn, and Ni at concentrations higher than 1 μg g^{-1} along with Mo. Except for the zinc and essential fatty acid supplements, all of the supplements from Company 4 contained Cu.

DISCUSSION

The high fidelity results obtained in this study were accurate and precise based on validation with SRM 3280. The microwave-enhanced sample preparation provided effective, consistent, and efficient sample decomposition that combined with the robust ICP-MS analysis demonstrated the feasibility of using EPA methods to routinely determine both xenobiotic and essential elemental contamination in dietary supplements. Only two other studies have used SRM 3280 to validate the method protocols employed for multi-element quantification in dietary supplements or botanicals (Avula et al., 2010, 2011). This study demonstrated superior analytical and statistical procedures compared with the above-mentioned two previous studies by Avula et al. through increased quality control and inclusion of CIs for all sample results. Another important improvement in the current study was related to the preparation of the calibration standards. The previous studies used a calibration blank solution of 3.2% HNO_3 and 0.8% HCl (Avula et al., 2010, 2011). Although these studies produced accurate results for SRM 3280, this method is not optimal in providing uniform acid content in all of the digested samples and calibration solutions. However, the addition of appropriate amount of analytical blank solution to each calibration standard during the subsequent dilution as performed in the current study accomplished this goal.

This study confirmed the presence of contamination from either xenobiotic or essential elements in all the samples from all four suppliers. The xenobiotic elements quantified in this study included Pb, Cd, Sb, Sn, and As, with their concentrations ranging from below 1 μg g^{-1} to μg g^{-1} levels. Only two supplements did not have xenobiotic elements detected in them, the zinc supplement from Company 1

and the essential fatty acid supplement from Company 2. Particularly alarming was the frequent presence of Al in μg g^{-1} quantities in half of the supplements tested. The most common sources of xenobiotic elemental contamination were V and U. Nearly two-thirds of the samples contained quantifiable amounts of Pb. Antimony was found in only one supplement at a concentration more than than 0.05 μg g^{-1}. One positive note was that the Hg concentrations were below the detection limit in all of the samples tested.

In addition to the presence of xenobiotic elements, most of the supplements contained essential elements such as Zn, Mg, Ca, Fe, Cu, Mo, and Se that were not listed as ingredients on the labels. This study identified a higher frequency of adulteration from essential elements compared with xenobiotic elements, with Ca being the most frequent contaminant. The zinc supplements from Companies 1 and 4 were the only two that did not contain quantifiable amounts of any extra essential element. Iron was almost always found in μg g^{-1} concentration as an impurity. The presence of either xenobiotic or essential elements in all of the dietary supplements raises the possibility of a systemic and persistent problem across the industry.

The contamination observed in this study was examined through a comparison of the four manufacturers. Company 3 had the highest occurrence of xenobiotic elemental content. Except for the essential fatty acid, each supplement contained at least five xenobiotic elements. It was also the only company that had four supplements with μg g^{-1} quantities of Al along with the two highest As concentrations. Their multivitamin supplement had the highest amount of As and V quantified in the study, while their zinc supplement had the second highest concentration of Pb. The elemental adulteration for Company 4 was nearly as frequent as Company 3. Their calcium supplement had the highest concentration of Al with the highest Sb concentration found in their zinc supplement. Company 1 had the least amount of xenobiotic content. However, its calcium supplement contained the highest concentration of Sn, and their zinc supplement had the second highest concentration of Cd. Both calcium and magnesium supplements from Company 1 had a similar pattern of xenobiotic contamination from Pb, Sn, U, and V. The company's use of the same ingredients in different amounts in their supplements may provide one explanation (Table 2). These supplement types from Companies 2 and 3 shared similar elemental adulteration but this was not observed in Company 4. Three of the five supplements from Company 2 had at least 10 μg g^{-1} of Al. The zinc supplement from Company 2 contained the highest concentration of Pb.

Some patterns appeared when the supplement types were compared. The essential fatty acids were the cleanest for xenobiotic elements as the products from Company 4 only contained over 3 μg g^{-1} of Al. The essential fatty acids were more likely to contain essential elements with Mg detected in all three samples analyzed. The zinc supplements were relatively clear of unlabeled essential elements, except for the one from Company 2 that contained Ca and Mg along with six other essential elements. The contamination frequency was less for the zinc supplement from Company 3, but it still included μg g^{-1} quantities of Ca and Mg. All of the calcium supplements were adulterated with at least 855 μg g^{-1} of Mg. The four magnesium supplements contained at least 198 μg g^{-1} of Ca and 15.8 μg g^{-1} of

Fe. Even though they had the most elements listed as ingredients, the multivitamins contained contamination from nearly all of the other essential elements, notably Fe at approximately 40 μg g^{-1} in all three. Both of the probiotics had over 200 μg g^{-1} of Mn. Certain bacteria are known to produce Mn, especially the *Lactobacillus* genus (Bomba et al., 2002), a common species in this type of supplement. The bacteria also need the other essential elements that were quantified in the probiotics for survival, especially Ca and Mg that were found in high concentrations. The media where the bacteria were grown is a possible contamination source.

The findings of this study are significant because they reveal the quality of dietary supplements in the current marketplace and the possible threat they pose to public health as their use increases globally. The long-term health effects from regular intake and exposure to elemental contaminants from dietary supplements are rarely studied and insufficiently understood. One of the less publicized aspects of these quality deficiencies is the presence of essential elements not mentioned on content labels, potentially negatively affecting supplement regimens designed to improve human health through nutrient support. With people taking multiple dietary supplements, potential negative synergistic effects are a realistic possibility and cumulative exposure to these xenobiotic elements may have long-term adverse health outcomes. In addition to the consequences from the cumulative exposure to these potentially harmful elements, over-supplementation can result from the presence of essential elements that were not listed on the label, as demonstrated by this study.

This study demonstrated the application of standardized EPA methods for the evaluation of elemental contamination and content in dietary supplements. The use of these methods resulted in finding xenobiotic and/or essential elemental contamination in all the dietary supplements analyzed. Their implementation in both sample preparation and analysis to determine elemental concentrations could serve as the foundation of a reliable, consistent, and efficient quality assurance program for the dietary supplement industry and regulatory agencies. The overwhelming trend that emerged from this study was the widespread contamination in the products from the different suppliers. The elemental adulteration observed could result from the manufacturing process or the raw ingredients that comprise the supplements. It is difficult to completely know their sources, but many ingredients, such as Stevia, come from countries where regulation is not as stringent as in the United States. It is clear from this study that in order to provide the highest quality of dietary supplements possible, all final products and ingredients must be analyzed for elemental contamination to ultimately meet the intent of the GMP standards of the Dietary Supplement Health and Education Act of 1994. Further studies are necessary to evaluate the type, level, and frequency of dietary supplement contamination throughout the industry.

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